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Thermogenic side effects to migratory predisposition in shorebirds

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Vézina F, Jalvingh KM, Dekinga A, Piersma T. Thermogenic side effects to migratory predisposition in shorebirds. *Am J Physiol Regul Integr Comp Physiol* 292: R1287–R1297, 2007. First published November 30, 2006; doi:10.1152/ajpregu.00683.2006.—In the calidrine sandpiper red knot (*Calidris canutus*), the weeks preceding takeoff for long-distance migration are characterized by a rapid increase in body mass, largely made up of fat but also including a significant proportion of lean tissue. Before takeoff, the pectoral muscles are known to hypertrophy in preparation for endurance flight without any specific training. Because birds facing cold environments counterbalance heat loss through shivering thermogenesis, and since pectoral muscles represent a large proportion of avian body mass, we asked the question whether muscle hypertrophy in preparation for long-distance endurance flight would induce improvements in thermogenic capacity. We acclimated red knots to different controlled thermal environments: 26°C, 5°C, and variable conditions tracking outdoor temperatures. We then studied within-individual variations in body mass, pectoral muscle size (measured by ultrasound), and metabolic parameters [basal metabolic rate (BMR) and summit metabolic rate (M_{sum})] throughout a 3-mo period enclosing the migratory gain and loss of mass. The gain in body mass during the fattening period was associated with increases in pectoral muscle thickness and thermogenic capacity independent of thermal acclimation. Regardless of their thermal treatment, birds showing the largest increases in body mass also exhibited the largest increases in M_{sum} . We conclude that migratory fattening is accompanied by thermoregulatory side effects. The gain of body mass and muscle hypertrophy improve thermogenic capacity independent of thermal acclimation in this species. Whether this represents an ecological advantage depends on the ambient temperature at the time of fattening.

muscle mass; summit metabolic rate; basal metabolic rate; thermogenic capacity; phenotypic flexibility

LONG-DISTANCE MIGRATION in birds is an energy-demanding phenomenon that leads to remarkable physiological adjustments that are among the best-known examples of phenotypic flexibility in higher vertebrates (52, 59). In birds preparing for long-distance flights, the period of migratory fattening often involves a reorganization of specific physiological systems, including metabolic changes ranging from the level of the cell (24, 42, 43, 44, 46, 65, 66) to that of the whole animal (6, 33, 39, 79). In the weeks preceding takeoff, as well as during a refueling stopover, birds have to maximize energy input to accumulate the fat stores necessary to fuel their nonstop flight. To do so, they enter a hyperphagic phase with extreme energy assimilation rates (34, 38, 60).

Although the migratory mass gain is made up of a large proportion of fat used as fuel (e.g., 10, 12, 19), it is now acknowledged that a significant amount of lean tissue is also

accumulated during this period (30, 32, 40, 41, 61). For example, in some shorebirds, the size of the nutritional organs increases significantly, growing early in the fattening period, to accommodate the elevated energy intake rate (7, 35, 52, 56). This is happening in parallel with the growth of the pectoral muscles, which, in preparation for endurance flight, usually reach a peak of mass independent of any training effect (21) during the days just before takeoff (35, 56). At departure time, pectoral muscles can form up to 35% of lean body mass in long-distance migrants (13).

Avian pectoral muscles are also of central importance in thermoregulatory processes under cold stress. To maintain body temperature at ambient temperatures (T_{as}) below thermoneutrality, birds rely mostly on shivering thermogenesis (20, 29, 47, 72). In small passerines species, cold acclimatization is thought to be mainly a metabolic process leading to enhanced shivering endurance (reviewed in Refs. 18, 19, 20, 47, 49, 69, 72) and may also include increases in pectoral muscle size (15, 50, 69). In contrast, because thermal conductance is negatively related to body mass (28) and because of seasonal adjustments in plumage, cold acclimatization in large species is thought to result mainly, if not exclusively, from changes in body insulation with little metabolic improvements (9, 20, 63, 67, 80).

Interestingly, several of the muscle metabolic adjustments found in birds acclimatizing to seasonal cold temperatures are also associated with endurance flight (reviewed in Refs. 19 and 72). Furthermore, recent research showed that captive medium-sized shorebirds (red knots *Calidris canutus islandica*) respond to experimental cold acclimation by maintaining a higher body mass, in association with larger pectoral muscles, in turn, leading to improved thermogenic capacity (78). Short-term body mass modulation in response to natural variations in weather conditions was also found in dunlin (*C. alpina*, see Refs. 16, 17, 31), suggesting that such adjustments of body mass may also be used as a form of cold acclimatization. On the basis of these arguments, one would expect “thermoregulatory side effects” to migratory fattening. That is, in birds fueling up for migration, the increase in body mass and the associated gain in muscle size would result in elevated thermogenic capacity, independent of cold acclimatization or acclimation. Depending on the climate where migratory fattening is taking place (i.e., tropics or temperate zone), this could or could not represent a functional advantage, i.e., a side benefit.

This paper investigates the side effect hypothesis, using captive red knots as a model, and presents data illustrating a new facet of phenotypic flexibility.

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MATERIALS AND METHODS

In this experiment, we followed captive red knots over a period including the complete fattening cycle beginning the first week of April, a month before the birds started gaining weight, and ending the last week of June, a month after resuming normal body mass (Fig. 1). All birds were weighed once a week during a routine feather molt scoring and health check. Once a month, we measured each parameter presented below over a period of 15 days, examining two individuals per day always in the same, randomly assigned, sequence (Fig. 1). This experiment complied with the Dutch Law on Experimental Welfare and the animal welfare guidelines of the Royal Netherlands Academy of Art and Sciences.

Experimental animals, diet, and treatments. Twenty-nine adult red knot of the subspecies *islandica* were used in this experiment (20 females, 9 males, PCR sexing; Ref. 1). The birds were captured in the Dutch Wadden Sea (53°31'N, 6°23'E) in September 2004 and brought into captivity initially in outdoor aviaries (4.5 × 1.5 × 2.3 m high) at the Royal Netherlands Institute for Sea Research (53°00'N, 4°47'E) and transferred later in January 2005 (i.e., 3 mo before the beginning of the present experiment) to controlled indoor aviaries of the same specifications (see Ref. 78). The birds had free access to fresh water for drinking and an artificial mudflat for probing. The floor of the aviaries was continuously flushed with salt water to minimize infections and skin lesions caused by dry feet.

The birds were maintained on a diet of mudsnails (*Hydrobia ulvae*) collected in the Wadden Sea. The snails used in the experiment were stored frozen, and freshly thawed portions were offered, in excess every day, in a tray filled with salt water. The thawed snails remained in their shells, so the birds had to crush the shells in their gizzard to digest the meat (e.g., 57, 77).

Treatment temperatures. We had five indoor aviaries to our disposal and we worked with three experimental treatments. Two groups of six birds were kept in aviaries ventilated with outdoor air, therefore tracking natural outdoor temperature. This treatment is called "variable." Birds kept in the variable treatment faced daily changes in T_a with a gradual seasonal increase during the experiment. From the first week of April to the last week of June, temperatures increased by 0.7°C per week from 13.1°C (SD 0.4) and 13.7°C (SD 0.4) in April to 22.1°C (SD 0.8) and 22.5°C (SD 0.9) in June for the two cages, respectively. Minimal and maximal temperatures experienced by the birds in the variable treatment during the experimental period were 10.7°C and 23.9°C, respectively. Two groups of six birds were

maintained at a constant T_a averaging at $26.4 \pm 0.5^\circ\text{C}$ and $26.0 \pm 0.5^\circ\text{C}$ for the two cages, i.e., within the zone of thermoneutrality (54, 81). This treatment is called the "warm" treatment. One group of six birds was maintained at an average temperature of $5.0 \pm 0.8^\circ\text{C}$, called "cold" treatment. We originally started the experiment with six birds in the cold treatment, but one individual died of unknown cause before the beginning of measurements. The use of statistical replicates for the variable and warm treatments allowed us to consider and control for potential group effect within treatment in our data (see below). All groups were comparable in terms of sex composition and morphometrics and were of comparable average structural body size (78). The light regimen in the cage was programmed to follow the natural photoperiod for the time of the year with gradual changes in luminosity (20 min) during the artificial "sunrise" and "sunset".

Respirometry. We measured basal metabolic rate (BMR), maximal thermogenic capacity [summit metabolic rate (M_{sum}); Ref. 74], as well as the temperature at which M_{sum} was reached (T_a at M_{sum}) as an indicator of heat loss compensation capacity. Although M_{sum} is not a sustainable state (27, 74), it is correlated with cold endurance (71, 75) and thus reflects the level of submaximal heat production that can be sustained for extended periods of time (37, 72). We used the respirometry setup and methods described by Piersma et al. (55) and Vézina et al. (78). Briefly, birds were fasted, with access to water, for 11 h and were then weighed to the nearest 0.1 g before being placed in a metabolic chamber for overnight BMR measurements (lasting 17 h and starting at 1600). Our O_2 and CO_2 analyzers were calibrated on a daily basis. Testing the system by calculating $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ from burning a known mass of pure alcohol in the chamber revealed that our system was accurate to 4% (Vézina F and Jalvingh KM, unpublished observation). During BMR measurements, the birds were maintained in the dark at 21°C (within the zone of thermoneutrality; Refs. 54, 81), and birds were weighed a second time at the end of the measurement session. Within 30 min following BMR measurements, the birds were placed in the metabolic chambers again for the measurement of M_{sum} . At this stage, we added a piece of 3-cm-thick rubber foam to the floor of the chamber to isolate the birds feet from direct contact with the cold chamber floor. We used the sliding cold exposure technique in a helium-oxygen environment (helox, 74) with M_{sum} trials starting at a T_a set to -15°C maintained for 30 min. The temperature was then decreased in decrements of 5°C each 30 min. We terminated a trial at first sign of hypothermia (falling metabolic rate over several minutes) or when a decrement in T_a produced no further increase in $\dot{V}\text{O}_2$ (74).

It should be acknowledged that measuring M_{sum} in fattening cold-acclimated birds pushed our equipment close to its working limit. Our temperature-controlled cabinet can generate temperatures (measured in the metabolic chambers) down to -30°C and some of the cold-acclimated birds were reaching M_{sum} at temperatures very close to that limit before the mass gain period in April (range of T_a at M_{sum} : -19.9 to -27.7°C). This suggests that our measured change in M_{sum} (see below) for cold-acclimated birds may be conservative, as T_a s below -30°C might have elicited an even higher heat production.

$\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ were calculated with the appropriate formulas for our setup, taking into account the presence of CO_2 in reference air, as described in Piersma et al. (55). We used the lowest and highest 10 min of $\dot{V}\text{O}_2$ measured in their respective trials as measures of BMR and M_{sum} , respectively (O_2 sampling interval: 30 s). Calculation of M_{sum} used the instantaneous measurements technique (3), while BMR calculations were based on the steady-state approach (55). Average respiratory quotient over all the trials was 0.71 ± 0.02 , indicating that the birds were using fat as an energy source during the experiments. Therefore, energy consumption was estimated using a constant equivalent of 20 kJ/l O_2 and then converted to watts (26, 53, 54, 55, 79). Calculations were performed with Warthog Systems LABANALYST X (Riverside, CA). We randomized the order in which birds from specific treatments were measured, and reported body mass was calculated as an average of first and second mass measured. One bird

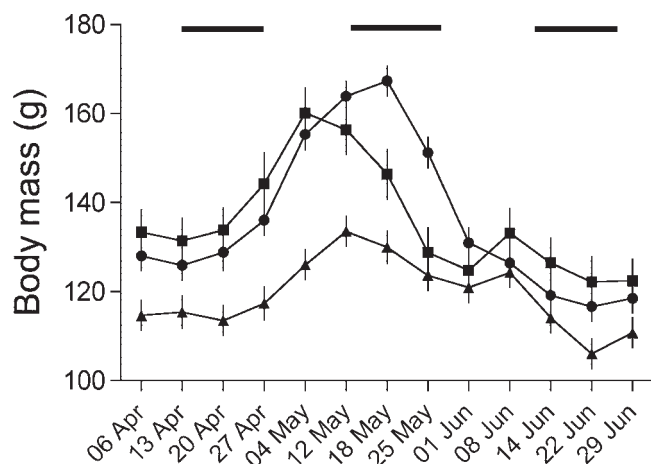


Fig. 1. Temporal variation in body mass in red knots acclimated to cold (squares), variable (circles), and warm (triangles) ambient temperatures (T_a s) during the period of migratory fattening. Horizontal bars indicate the periods of metabolic measurements. Muscle thickness was measured on each bird a day before the beginning of this period.

Table 1. Repeated-measures ANOVA considering time and treatment effect on body mass, muscle thickness, and the temperature eliciting maximal heat production

Independent Variable	Dependent Variable								
	Body mass at moult score			Muscle thickness			T _a at M _{sum}		
	df	F	P	df	F	P	df	F	P
Date	12, 298	25.5	<0.0001	2, 52	8.6	<0.005	2, 43	6.1	<0.01
Treatment	2, 2	10.7	0.08	2, 2	0.1	0.9	2, 2.1	3.8	0.2
Cage (treatment)	2, 25.7	1	0.4	2, 24	8.5	<0.005	2, 23.9	1.3	0.3
Bird [cage (treatment)]	26, 298	10.9	<0.0001	25, 52	1.4	0.2	23, 43	3.8	<0.0001
Treatment × date	24, 298	3.5	<0.0001	4, 52	3.3	<0.05	4, 43	1.8	0.1

df, Degrees of freedom. *P* values in bold are referred to in the text.

from the warm treatment was clearly restless during the June BMR measurement and was omitted from the analysis for that month. In some cases, we had birds showing minor toe frostbite problems after the first helox measures. These birds were not used in the further helox trials to avoid any additional foot problems. Therefore, our sample size has a slight unbalance between months regarding BMR and M_{sum}.

Ultrasonography. The use of noninvasive ultrasonography allows for repeated measurements of organ size on the same individuals. Using this technique, each month we measured the thickness of the pectoral muscle with an ultrasound scanner (model AQUILA; Pie Medical Benelux, Maastricht, The Netherlands) using an 8-MHz linear probe and ultrasonic gel to make contact with the animal skin. Measurements were made according to the technique presented in Dietz et al. (22) and Lindström et al. (40) and were performed with the observer being blind to experimental treatment for specific birds. Pectoral muscle size is presented as muscle thickness (cm) measured from the skin to the sternum. Preliminary trials with this apparatus and observer (A. Dekinga) revealed high repeatability of the measurements (calculated according to Lessells and Boag, Ref. 36; $r = 0.97$).

Statistical analysis. In this experiment, we were interested in the effects of the changes in body mass and muscle thickness on changes in metabolic parameters during the period of migratory mass gain and loss. Total body mass includes muscle mass, and these variables are therefore correlated (40, 78). Hence, it may be difficult to detect effects that are specific to body mass or muscle thickness only. We nevertheless performed the analyses using these two variables separately, because of the predominant role of muscles in shivering heat production, with the aim to potentially identify different muscle or total mass-specific patterns. Migratory fattening in red knots happens in May (Fig. 1). We therefore calculated the change in mass, muscle thickness, and metabolic variables from April to May (mostly positive changes) and from May to June (mostly negative changes), providing two time periods (Fig. 1). Employing a repeated-measures ANCOVA approach (univariate mixed general linear model), we studied how the measured parameters covaried with the changes in either body mass or muscle thickness over the whole period, while taking treatment effects and potential interactions into account. We considered a possible group effect in all of the analysis by including the variable "cage" nested in "treatment." Furthermore, we controlled for the time period effect, and thus the repeated nature of the analysis by including the independent variable "time period" and its interaction with mass or muscle thickness into the model. Additionally, we controlled for potential individual effects by including "bird id" nested in "cage" nested in "treatment" in all analysis. When finding a significant interaction, we split the analysis by either time period or treatment. When interactions were nonsignificant, we removed them from the model. Using this method, we investigated relationships, without forcing through the origin, between changes in mass and pectoral muscle thickness and changes in BMR, M_{sum}, and T_a at M_{sum} from April to June. All data were tested for normality using a Kolmogorov-

Smirnov test before performing the analyses. Data are presented as means ± SE.

RESULTS

Changes in body mass and muscle thickness. Analysis of our weekly mass data revealed a strong pattern of mass change over time, as well as a time-specific treatment effect on body mass (date: $F_{12,298} = 25.5$, $P < 0.0001$; date by treatment interaction: $F_{24,298} = 3.5$, $P < 0.0001$; Table 1). Indeed, birds from all treatments gained mass during the period of migratory fattening from the last week of April to mid-May (Fig. 1). However, the relative increase depended on the experimental treatment with mass rising by 16.5, 20.1, and 30.7% in the warm, cold, and variable treatments, respectively. The peak of body mass also differed in time between treatments with the cold-acclimated birds reaching their maximal mass first followed by warm- and variable-acclimated individuals (Fig. 1). Two weeks after the latest peak in mass, birds from all treatments had resumed a nonmigratory body mass.

The variation in muscle thickness recorded during the fattening period mirrored the changes in mass. As with body mass, we found a significant time effect, as well as a time-specific treatment effect on muscle thickness (date: $F_{2,52} = 8.6$, $P < 0.005$; date by treatment interaction: $F_{4,52} = 3.3$, $P < 0.05$; Table 1). This resulted in birds from all treatments showing an increase in muscle thickness, with the actual average change depending on the specific thermal treatment (warm: +3.3%; cold: +7.9%; variable: +14.3%). Repeated-measures analysis revealed that, over the whole experimental period, there was a direct relationship between the change in body mass and the change in pectoral muscle thickness ($F_{1,27} = 26.6$, $P < 0.0001$; Fig. 2, Table 2) but that this relationship did not differ between treatments or time periods ($P > 0.5$ in both cases; no significant interaction terms). Therefore, for a given change in body mass, the associated change in pectoral muscle size was not affected by the thermal treatment.

Changes in metabolic parameters in relation to changes in body mass and muscle thickness. Repeated-measures ANCOVA revealed a highly significant effect of the change in mass on the change in M_{sum} ($F_{1,20} = 18.5$, $P < 0.0001$; Fig. 3A, Table 2), suggesting that the capacity to improve heat production was closely related to variations in body mass. This relationship was independent of the thermal treatment and time period ($P \geq 0.6$, in both cases) and no significant interaction terms were detected. Therefore, with respect to body mass changes, the enhancement in the capacity to produce heat

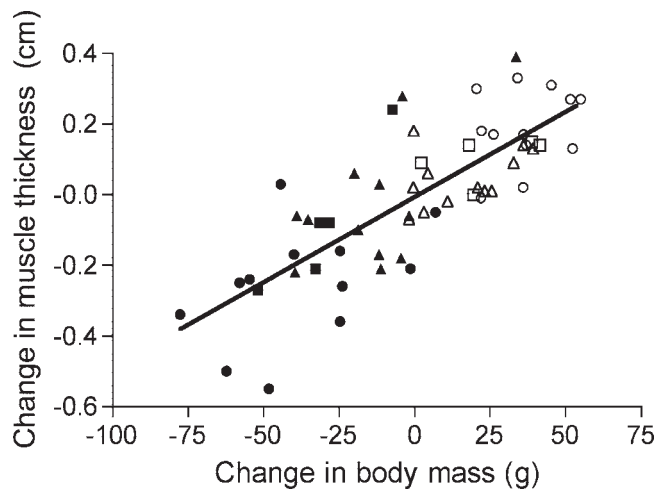


Fig. 2. Repeated-measures regression showing the relationship between the change in pectoral muscle thickness and the change in body mass across treatments for the periods of gain (white symbols) and loss (black symbols) of body mass. Each individual is represented twice in the cloud of points. Squares, cold; circles, variable; triangles, warm.

associated with a given increase in body mass was not affected by whether the birds were acclimated to cold or warm conditions. The change in thermogenic capacity also appeared to be related to the change in muscle thickness, independently of thermal treatment or whether the birds were accumulating or losing mass (Fig. 3B, Table 2). However, this relationship did not pass the 0.05 threshold of statistical significance when considering the other effects in the model ($P = 0.09$; no time period or interaction effects). In this particular case, there were not enough degrees of freedom to compute treatment effects. We therefore re-ran the analysis, removing the nonsignificant cage effect from the model. In this second analysis, the relationship between the change in muscle thickness and the change in M_{sum} remained nonsignificant ($P = 0.09$), and thermal treatment had no significant effect ($F_{1,25} = 0.2$, $P = 0.8$).

Repeated-measures ANCOVA revealed that the changes in BMR taking place over the period of gain and loss of mass were closely related to the simultaneous changes in overall body mass or muscle thickness. The specific effect of the change in body mass on the change in BMR, however, differed between birds that were either gaining or losing mass (significant interaction time period by change in mass; $F_{1,25} = 12.1$, $P < 0.005$, Table 3). We therefore analyzed these two periods separately. During migratory fattening, the relationship between the change in mass and the change in BMR was not significant when taking treatment and cage effects into account ($P = 0.3$, no significant interaction terms Fig. 4A). The change in BMR was affected by thermal treatment, however ($F_{2,12} = 5.8$, $P < 0.05$), but this effect was weak, as post hoc sequential Bonferroni analysis detected no significant differences between treatments. During the period of mass loss, the change in BMR was clearly affected by the change in mass ($F_{1,22} = 11.5$, $P < 0.005$, Fig. 4B), independently of any treatment effect ($P = 0.9$, no significant interaction term). Therefore, variations in body mass had little effect on the variations in BMR during migratory fattening, but when the birds resumed a normal body mass, the actual decreases in mass was accompanied by a decrease in BMR.

Table 2. Repeated-measures ANCOVA testing relationships between change in muscle thickness and helox metabolic variables and changes in body mass or muscle thickness

Independent Variable	Change in muscle thickness			Change in M_{sum}			Change in T_a at M_{sum}		
	df	F	P	df	F	P	df	F	P
Time period	1, 27	0.2	0.6	1, 20	0.9	0.4	1, 19	22.4	<0.0001
Treatment	2, 22	0.9	0.5	2, 13	0.9	0.6	2, 22	1.7	0.4
Cage (treatment)	2, 24.5	1.0	0.4	2, 38.3	1.4	0.3	2, 26.3	2.6	0.1
Bird [cage (treatment)]	24, 27	0.7	0.8	21, 20	0.2	1.0	21, 19	1.7	0.1
Change in body mass (A)	1, 27	26.6	<0.0001	1, 20	18.5	<0.0001	1, 19	10.7	<0.005
Change in muscle thickness (B)							1, 19	15.3	<0.005
†Time period \times A or \times B			n.s.			n.s.			n.s.
‡Treatment \times A or \times B			n.s.			n.s.			n.s.
Total R^2		0.78			0.72			0.78	
							‡analysis split by treatment		
							‡analysis split by time period		

*Not enough degrees of freedom to compute treatment effect (see text for detail). †Interaction tested depends on the covariate included in the model (change in body mass or change in muscle thickness).

‡In case of significant interactions, the analyses have been split by time period or treatment. See text for results. P values in bold are referred to in the text.

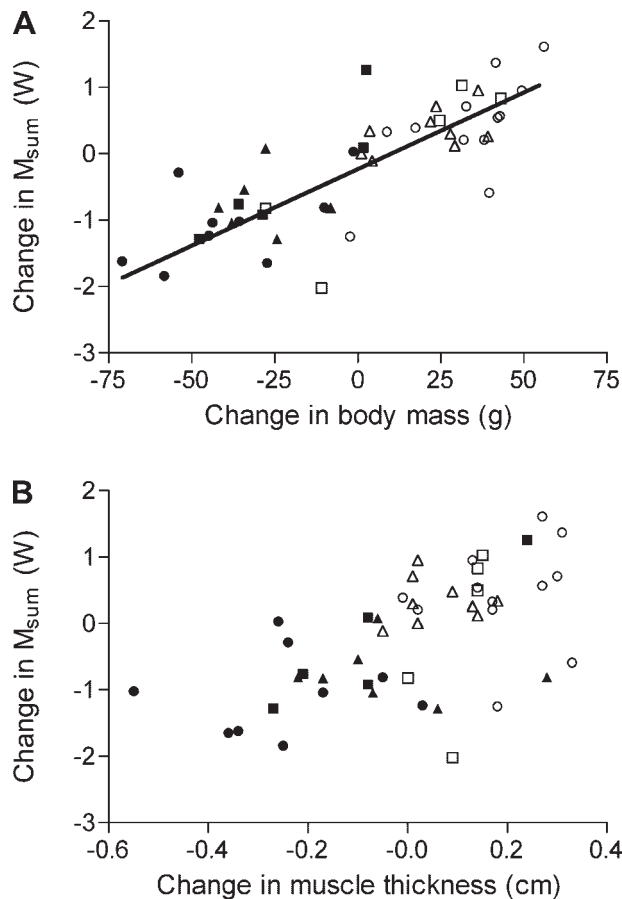


Fig. 3. Repeated-measures regression showing the relationship between the change in maximal thermogenic capacity, (summit metabolic rate M_{sum}), and the change in body mass (A), and the change in muscle thickness (B) across treatments for the periods of gain (white symbols) and loss (black symbols) of body mass. Each individual is represented twice in the cloud of points. Squares, cold; circles, variable; triangles, warm.

The analysis investigating relationships between changes in muscle thickness and change in BMR revealed no specific time period, thermal treatment, or interaction terms effects. Yet, across treatment, the change in BMR was positively associated with the changes in pectoral muscle thickness ($F_{1,26} = 9.3$,

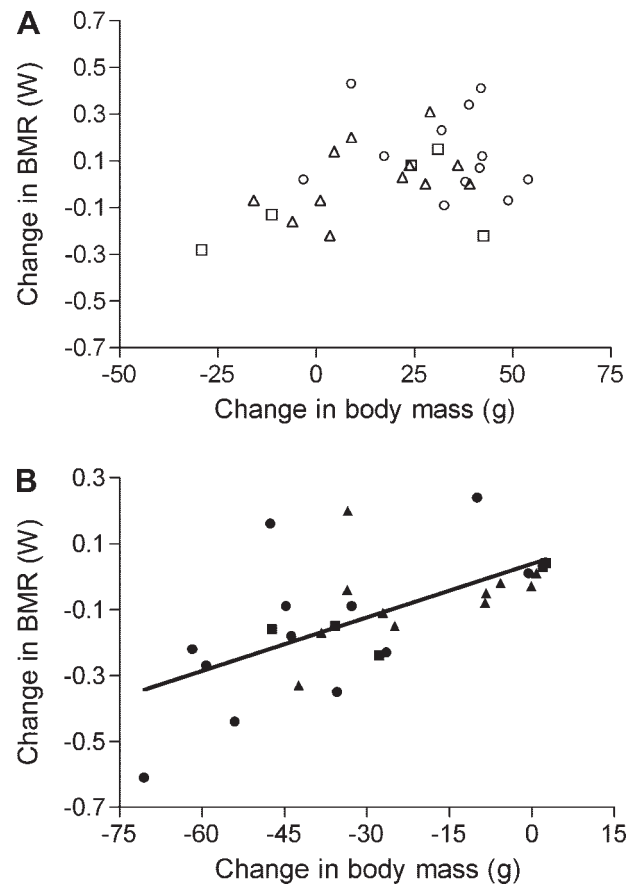


Fig. 4. Relationship between the change in basal metabolic rate (BMR), and the change in body mass across treatments for the periods of gain (A) and loss (B) of body mass. Squares, cold; circles, variable; triangles, warm.

$P < 0.01$; Fig. 5, Table 3). Therefore, the gain and loss of pectoral muscle tissue was associated with an increase and decrease of BMR.

Temperature of maximal heat production. Investigating the relationship between the change in body mass and the change in T_a at M_{sum} over time revealed a significant treatment by change in mass interaction term ($F_{2,17} = 6.7$, $P < 0.01$; Table 2). We therefore segmented the analysis per treatment. For the

Table 3. Repeated-measures ANCOVA testing relationships between change in BMR and change in body mass or muscle thickness

Independent Variable	Dependent Variable					
	Change in BMR			Change in BMR		
	df	F	P	df	F	P
Time period	1, 25	3.8	0.06	1, 26	0.04	0.8
Treatment	2, 33.3	13.9	<0.0001	2, 2.2	2.5	0.3
Cage (treatment)	2, 24.1	0.01	1.0	2, 25.1	0.8	0.4
Bird [cage (treatment)]	24, 25	0.9	0.6	24, 26	0.4	1.0
Change in body mass (A)	1, 25	14.9	<0.005			
Change in muscle thickness (B)				1, 26	9.3	<0.01
*Time period \times A or \times B	1, 25	12.1	<0.005			n.s.
*Treatment \times A or \times B			n.s.			n.s.
Total R^2		0.69			0.53	
†analysis split by time period						

*Interaction tested depends on the covariate included in the model (change in body mass or change in muscle thickness). †In case of significant interactions, the analyses have been split by time period or treatment. See text for results. P values in bold are referred to in the text.

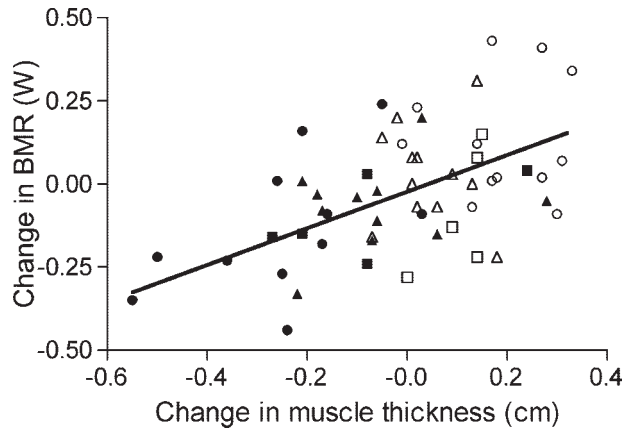


Fig. 5. Repeated-measures regression showing the relationship between the change in BMR, and the change in muscle thickness across treatments for the periods of gain (white symbols) and loss (black symbols) of body mass. Each individual is represented twice in the cloud of points. Squares, cold; circles, variable; triangles, warm.

cold- and variable-acclimated birds, the change in T_a at M_{sum} was not significantly related to changes in body mass when considering time period and individual effects (cold: $P = 0.6$, variable: $P = 0.9$; Fig. 6, A and B). In warm-acclimated birds, however, there was a clear relationship between changes in body mass and changes in T_a at M_{sum} ($F_{1,5} = 8.3$, $P < 0.05$; Fig. 6C). This relationship, therefore, implies that in birds acclimated to thermoneutral conditions, the gain in mass allowed them to reach their maximal heat production at lower helox temperatures. Indeed, when plotting T_a at M_{sum} for the three treatments against month (Fig. 7), one can see that warm-acclimated birds at peak of mass (May) were comparable to the other treatments for the same period. However, although there was a clear time effect (date: $F_{2,43} = 6.1$, $P < 0.01$, Table 1), this interaction of treatment by date did not reach significance ($P = 0.1$).

Comparing changes in pectoral muscle thickness with changes in T_a at M_{sum} , revealed an overall across-treatment negative relationship between these variables ($F_{1,19} = 10.7$, $P < 0.005$; Table 2). This indicates that, overall, birds with larger muscles were reaching their maximal thermogenic capacity at lower helox temperatures. However, this correlation was also influenced by the time period (time period by change in muscle thickness interaction: $F_{1,19} = 15.3$, $P < 0.005$; Table 2). Restricting the analysis within the period of mass gain or mass loss revealed no such relationship ($P > 0.2$, in both cases), but significant treatment effects (period of mass gain: $F_{2,7} = 7.7$, $P < 0.05$; period of mass loss: $F_{2,3} = 16.6$, $P < 0.05$), indicating that within mass gain or mass loss periods, T_a at M_{sum} changed differently among treatments (see Fig. 7).

DISCUSSION

Our results demonstrate that independent of any thermal acclimation, the migratory increases in body mass in red knots are associated with increases in maximal thermogenic capacity, an indicator of cold endurance (71, 75). The consequence of this change in body mass was most visible for birds acclimated to thermoneutral conditions, although they exhibited the smallest gain of body mass (17% relative to 20–31%).

Changes in body mass and pectoral muscles and their effects on thermogenic capacity. Birds from all treatments showed the typical cycle of mass gain and loss previously reported for captive knots in preparation for long-distance migratory flight (e.g., see Refs. 51, 54, 58). The actual change in mass, however, was affected by thermal conditions. While cold- and variable-acclimated birds showed peaks of mass typical of captive *islandica* knot (160–167 g; compare with Ref. 58), warm-acclimated birds showed a surprisingly low level of fattening with average body mass peaking at 134 g. The reasons behind this thermal treatment effect on the level of

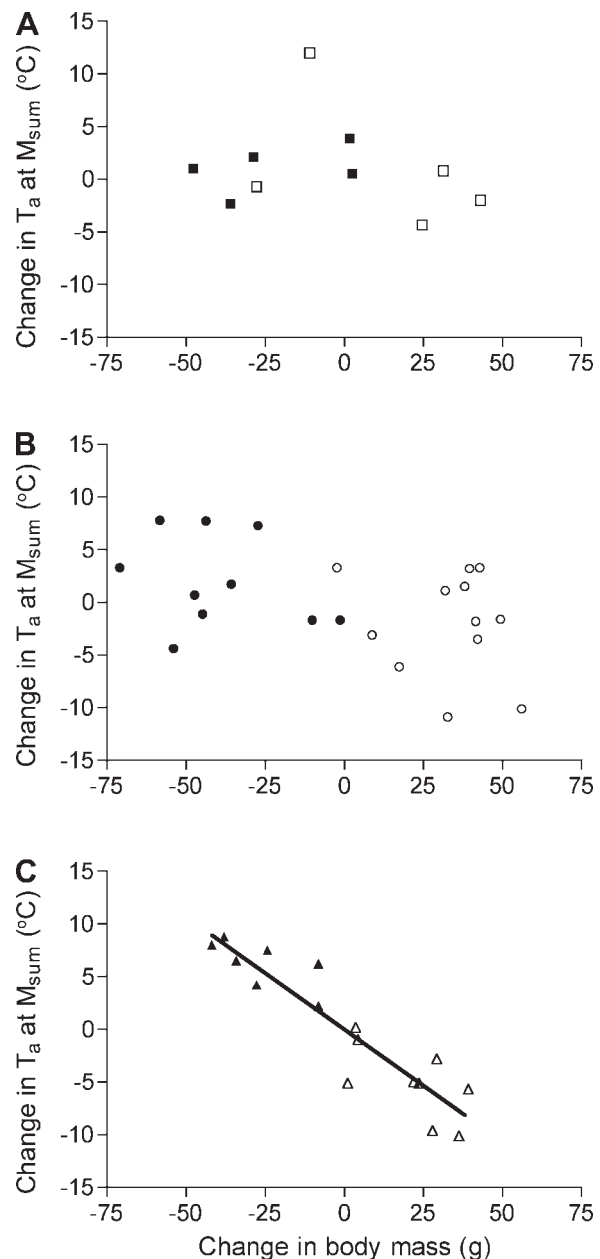


Fig. 6. Repeated-measures regression showing the relationship between the change in the measured temperature inducing maximal thermogenic capacity (T_a at M_{sum}) and the change in body mass in cold (A), variable (B), and warm-acclimated (C) red knots for the periods of gain (white symbols) and loss (black symbols) of body mass. Each individual is represented twice in the cloud of points.

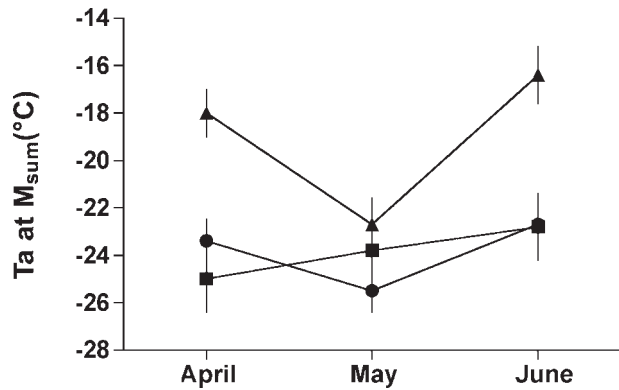


Fig. 7. Monthly T_a s at which maximal thermogenesis was induced in helox-exposed birds from three acclimation groups. Squares, cold; circles, variable; triangles, warm.

fattening are not clear. Living at thermoneutrality, warm-acclimated knots did not have to spend energy in heat production and could therefore have benefited from that “economy” by building larger fat stores but did not seem to do so.

There are two potential explanations for this phenomenon. First, even when not considering the chilling effects of the wind, free-living *islandica* knots fattening in the south Wadden Sea in April and May typically experience average daily temperatures of 11–14°C (average daily air temperature calculated from 1991 to 2001; Royal Netherlands Meteorological Institute, Den Helder station). More precisely, the long-term average daily T_a for the period of recorded gain of mass in our warm-acclimated birds (April 20th to May 12th) is $12.3 \pm 3.3^\circ\text{C}$. That is 12.7°C below the actual temperature experienced by our captive warm-acclimated birds. It is possible that this temperature discrepancy has affected the perceived physiological cue triggering fattening. Therefore, birds acclimated to thermoneutral conditions may have responded only partially with a lower fat and lean mass gain compared with birds from the other thermal treatments. Alternatively, a T_a of 25°C may be constraining for knots in the fattening period. Recent evidence has shown that subspecies of red knots overwintering in the tropics have low rates of fattening compared with subspecies fueling at higher latitudes (60). It has been suggested that this could be a strategic response to heat-load problems resulting from the combination of solar radiation and larger heat-producing internal organs (8). For instance, knots facing severe heat conditions in northwest Australia apparently maintain a small digestive tract to reduce internal heat production due to heat increment of feeding and thus cannot fuel as fast (8). Such temperature effects on the gain of mass have never been tested experimentally among species nor intraspecifically.

According to previous findings (21, 40), the variation in body mass in our birds was correlated with changes in the size of the pectoral muscles within individuals. Interestingly, this relationship was independent of thermal treatment, meaning that regardless of thermal acclimation, a given increase in body mass resulted in the same increase in pectoral muscle size for all birds. Accordingly, the recorded effect of the change in body mass on the variation in thermogenic capacity was not different between treatments. Across the measured range of body mass change, the change in M_{sum} varied from -2 W to $+1$ W (see Fig. 3A). With M_{sum} averaging between 6 and 7 W

in these birds (i.e., $7.5 \times \text{BMR}$; see Ref. 78), the recorded maximal increase in M_{sum} represents an individual capacity to improve heat production by up to 14–16% (assuming a 1-W increase in M_{sum}). These values are comparable to the 13% difference in thermogenic capacity recorded earlier as the result of cold acclimation in these same individuals (78).

Because they form the largest muscle group in the avian body (22% of total lean body mass in red knots; see Ref. 53), pectoral muscles are considered the main thermogenic organs in birds (18, 19, 20, 47, 72). However, when statistically considering the repeated nature of the data, as well as individual effects, the relationship between the change in pectoral muscle thickness and the change in M_{sum} remained marginally nonsignificant ($P = 0.09$). It is important to realize, however, that shivering heat production also occurs in other important groups of muscles, such as thigh muscles (14, 45). Furthermore, dissection of migratory red knots at various fattening levels showed that thigh muscle mass, as well as carcass mass (including all other skeletal muscles other than the pectoral), increase during the migratory body mass gain (56). Thus, our data only provide information for part of the heat-producing machinery. Since the measured change in pectoral muscle thickness was related to the change in body mass within individuals, we therefore consider it reasonable to assume that size changes in pectoral and other muscles played an important role in the recorded variation in thermogenic capacity during the fattening period.

Increasing maximal thermogenic capacity means that individuals can compensate larger gradients between air and body temperature, therefore reaching M_{sum} , or a lower submaximal sustainable level of heat production (75), at lower T_a . In red knots, cold acclimation is associated with larger body mass and muscle thickness, both negatively correlated to the T_a at which maximal heat production is reached (78). Therefore, given the results discussed above, one would predict that all birds increasing in body mass would experience an improvement in thermogenic capacity, independently of thermal treatment, and thus reach their maximal heat production at colder T_a s. Surprisingly, although birds from all treatments showed increases in body mass, with correlated variation in pectoral muscle size and M_{sum} , the relationship between changes in body mass and changes in T_a at M_{sum} was only significant for warm-acclimated birds (see Fig. 6). This result translated in these individuals reaching their maximal thermogenic capacity during peak of fattening at temperatures comparable to the ones recorded for variable and cold-acclimated individuals (see Fig. 7).

The reasons for this treatment effect on the change in T_a at M_{sum} are not clear. In cold-acclimated individuals, it may be that the cooling limit of our system (-30°C) prevented us from measuring a large change in T_a at M_{sum} , explaining why the variation was limited to $\pm 5^\circ\text{C}$ in this group (Fig. 6A). In birds acclimated to variable conditions, however, the measured range of variation in the change in T_a at M_{sum} , was very similar to the variation recorded for warm-acclimated individuals (-10°C to $+10^\circ\text{C}$ Fig. 6, B and C). Yet, the relationship with change in body mass remained nonsignificant in birds experiencing variable conditions, when considering individual effects and repeated measures. A likely explanation for this finding is provided by a recent study performed by Dietz et al. (23). Using dissection data collected over 21 years (155 *islandica*

knots) and flight tests in captive *islandica* knots, they showed that during active fattening, there is a break point in the relationship between body mass and pectoral muscle mass in this species (at body mass = 148 g). This indicates that, beyond this point, the rate of fattening is higher than the actual rate of pectoral muscle growth (i.e., the slope of the relationship between pectoral muscle mass and body mass is shallower). Over 160 g body mass, the birds even encounter maneuverability problems in flight because the size of their flight muscles does not match the requirements for constant relative flight power. In our study, both the variable- and cold-acclimated groups increased their body mass to or above the 160-g limit (peak body mass; variable: 167.4 g, cold: 160.2 g, see Fig. 1). Warm-acclimated individuals, however, showed the smallest change in body mass during fattening, peaking at 133.6 g. Thus, even at peak of fattening, warm-acclimated birds maintained their average body mass below the 148-g break point, suggesting that the change in body mass in these individuals resulted in a relatively larger change in pectoral muscle thickness. This explains the observation that, although warm-acclimated individuals only had a modest change in body mass, they exhibited the largest change in heat loss compensation (as visible in Fig. 6C). Interestingly, this 17% average increase in body mass is comparable to the actual difference in body mass resulting from cold acclimation (14–15%) in this group of birds (see Ref. 78, and compare differences between treatments for April and June in Fig. 1).

Increases in lean components of body mass in response to cold acclimation or acclimatization have been found in other small- to medium-sized birds. Although some studies reported no seasonal difference in lean body mass (e.g., see Refs. 15 and 68), increases in total lean mass in winter compared with summer have been reported for the house sparrow (*Passer domesticus*, Refs. 2 and 11) and house finch (*Carpodacus mexicanus*), in which case pectoral muscles were also larger in winter (50). Dunlins wintering in Britain show higher total lean mass, mostly pectoral muscles, in colder estuaries (16, 17). Swanson (68) reported larger pectoral muscles and livers in cold-acclimatized dark-eyed juncos (*Junco hyemalis*), whereas desert-dwelling hoopoe larks (*Alaemon alaudipes*) exhibited larger liver, kidney, and intestines when acclimated to cold conditions (82). Furthermore, Cooper (15) reported larger pectoral muscles in mountain chickadee (*Poecile gambeli*) and juniper titmice (*Baeolophus griseus*) in association with improved thermogenic capacity.

Swanson and Olmstead (76) suggested that in passerines, “increases in muscle mass devoted to shivering thermogenesis might be a mechanism for short-term adjustments of metabolism to temperature.” Since enlargements of pectoral muscles in response to cold conditions were also found in nonpasserine species, such as red knots and dunlins (16, 17, 78), it could be that modulation of total body mass, including the shivering muscle machinery, is actively used in response to variations in thermal environment in small- to medium-sized birds. Accordingly, Kelly et al. (31) showed that body mass in captive wintering dunlins increases in days with high wind speed and low T_a s. Swanson (71) showed intraspecific relationships between body mass and maximal thermogenic capacity in black-capped chickadee (*Poecile atricapillus*) and dark-eyed juncos. Here, we show that intraindividual variation in body mass, although happening for another purpose than cold acclimation,

is also related to variation in M_{sum} . Given that pectoral muscle mass tracks changes in total body mass in red knots (21, 40), it is thus possible, at least for the *islandica* subspecies, which may experience relatively low T_a during fattening, to take advantage of transient increases in body mass in terms of improved capacity to generate heat. Whether the increase in thermogenic capacity appearing during migratory fattening confers an ecological advantage depends on the T_a at the time of fattening. In the case of the *islandica* subspecies, this phenomenon could be adaptive, especially during southward migration when fattening occurs in the Arctic.

Variations in BMR during the fattening period. The fattening period in migratory birds is associated with significant accumulation of protein (41). In red knots, part of this lean tissue growth is not only due to the enlargement of the pectoral muscles (21, 40), but also to the development of other muscles and internal organs such as heart, liver, and kidneys (56). Muscles have relatively low energy consumption at rest compared with other metabolically active internal organs (25, 62, 64). However, a significant accumulation of lean metabolic tissue is likely to have a measurable impact on the overall energy consumption of resting birds, thus elevating BMR during fattening (53, 54, 79) and generating within-individual changes in BMR (33). Accordingly, we found correlated variation between the change in BMR and the change in body mass and muscle thickness in fattening knots. However, the effect of the change in body mass on BMR variation was only significant when birds resumed a nonmigratory state. This may be due to the relative amount of fat tissue accumulated during the period of mass gain. Fatty tissues have low metabolic activity (64), and, in a group of animals differing in the size of their fat stores, fat can confound the effect of body mass on BMR by increasing total body mass in individuals with a relatively small amount of lean tissues. In red knots, fat can make up to 77% of the mass gain during refueling (see Table 1 in Ref. 56). Thus, individual variation in the rate of fattening (i.e., within-individual rate of fat accumulation vs. lean tissue accumulation) may have generated a “diluting effect” in our data during the period of mass gain.

The return to nonmigratory body mass in migratory and captive shorebirds is accompanied by substantial decreases in the size of virtually every internal organ (4, 5, 6, 79). Thus, it is perhaps not surprising to find a tighter relationship between the change in body mass and the change in BMR in our birds for this period (Fig. 3B). We believe that BMR simply varied with the amount of lean tissue, independent of thermal acclimation. This hypothesis is supported by the relationship between the change in BMR and the change in pectoral muscle thickness.

Thermogenic side effects to migratory predisposition. We are aware of only two other studies investigating thermogenic capacity in birds in migratory disposition (70, 73). Swanson (70) and Swanson and Dean (73) reported higher thermogenic capacity during spring migration compared with other seasons in four species of passerine birds. Similarly, higher BMR was found in association with elevated M_{sum} in migratory yellow-rumped warbler (*Dendroica coronata*, see Ref. 73). These studies supported the idea of an elevated M_{sum} in migratory birds as a by-product of the physiological adjustments for endurance flight (70, 72, 73). Although they did not investigate within-individual variation or changes in muscle size per se,

they did suggest an increase in pectoral muscle mass as a potential underlying metabolic adjustment.

In contrast to research on small migratory birds, Saarela and Hohtola (63) compared summer- and winter-acclimated captive pigeons and showed that heat production under various cold stresses was not affected by an active (flying in aviary) or sedentary (restrained in cages) way of life. Winter birds had higher body mass with correlated pectoral muscle mass and better insulation reflected by a lower thermal conductance. Insulation was considered the main adjustment to cold conditions. Interestingly, winter plumage in red knots offers 1.35 times more insulation than summer plumage (54). Therefore, enhanced winter insulation together with a higher body mass, as visible in natural free-living conditions (see Fig. 33 of Ref. 51) and experimental cold-acclimated conditions (78), suggest that *islandica* red knots use a combination of improvement in plumage and thermogenic capacity to face their relatively harsh winter habitat (see Ref. 81).

Winter acclimatization could also result in adjustments at the tissue level (e.g., see Refs. 48 and 70). However, our data do not allow for investigation of such a possibility. Nevertheless, it should be noted that Weber and Piersma (79) found no significant changes in maximum tissue respiration in association with migratory mass changes in red knots (cytochrome *c* oxidase activity measured in heart, liver, and kidney, as well as flight and leg muscles). Similarly, Selman and Evans (65) found no changes in mean activity of succinate dehydrogenase in the liver and heart of red knots when comparing fattening and nonmigratory birds. The activity of that enzyme, however, increased by 80% in the small intestine and decreased by 60% in the pectoral muscle during hyperphagia. These authors postulated that this was an energy compensation strategy, allowing to counterweigh the costs of highly active nutritional organs and that the phenomenon should be reversed later at peak of mass when the digestive organs atrophy and the pectoral muscles rapidly expand (56).

Within individuals and independent of thermal acclimation, variation in overall thermogenic capacity was positively correlated with changes in body mass during migratory fattening in red knots. The growth of the pectoral and other muscles (see Ref. 56) in preparation for sustained exercise is most likely responsible for the improved maximal heat production. Although birds from all treatments showed mass-dependent increases in thermogenic capacity at peak of fattening, the effect on heat loss compensation was the most obvious in warm-acclimated birds despite their modest increase in body mass. Our data therefore highlight thermogenic side effects to the gain of mass in knots fueling up for migration. Cold acclimation per se, is thus not a prerequisite for improved maximal thermogenic capacity in this species.

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